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Protection by attenuated simian immunodeficiency virus in macaques against challenge with virus-infected cells

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A vaccine against AIDS will probably have to protect against challenge both by viable virus-infected cells and by cell-free virus. Eight cynomolgus macaques infected with attenuated simian immunodeficiency virus (SIV) were challenged (four each) with cell-free and cell-associated SIV. All were protected, whereas eight controls were all infected after challenge. These findings show that live-attenuated vaccine can confer protection against SIV in macaques. Extrapolation to human beings will require extensive evaluation of the safety of attenuated retroviruses. Alternatively, the mechanism of this potent protection must be understood and reproduced by less hazardous means.

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A major problem in the development of an effective AIDS vaccine is that HIV-1 may be transmitted by virus-infected cells as well as by free virus particles. The infection of macaques with simian immunodeficiency virus (SIV) is a model for HIV infection in man. In this model immunisation with subunit or recombinant envelope immunogens cannot elicit antiviral immunity that prevents infection, except with homologous cloned SIVmne.[1] Daniel et al[2] demonstrated that chronic infection with a molecularly engineered, attenuated clone of SIVmac239 prevented superinfection with homologous uncloned cell-free virus stocks of SIVmac. Using independently constructed clones, we studied whether different attenuated strains of SIV confer protection against pathogenic isolates and whether such protection is effective against cell-associated as well as cell-free virus challenge.

Two molecular clones of SIV, called J5 and C8, have been isolated. They are identical in sequence, except for seven differences located in the *nef* gene or the 3' long-terminal-repeat. One of these differences is a 12 basepair deletion, in C8, where the *nef* gene overlaps the U3 region of the repeat.[3] We have found by PCR and the persistence of anti-SIV antibodies that J5 and C8 viruses can infect cynomolgus macaques chronically. However, the C8 virus expresses an attenuated phenotype in vivo. 2 weeks after infection, virus is readily reisolated from the blood of C8-infected or J5-infected animals, but the proportion of infected lymphocytes is 10–100 times lower in the former. By 8–12 weeks, reisolation of C8 virus becomes sporadic and mean antibody titres are 10-fold lower in C8-infected than in J5-infected macaques. None of the C8-infected animals has developed AIDS-like disease even after 2 years (ref 3 and our data).

Four purpose-bred macaques (L103–L106) were injected intravenously with 10^4 median tissue-culture infective doses ($TCID_{50}$) of a titrated stock (from the 9/90 pool) of C8 grown in the human T-cell line C8166.[3] All macaques became infected. Although virus was rarely isolated by co-cultivation of C8166 cells with 10^7 peripheral blood mononuclear cells after 8 weeks, proviral DNA was repeatedly detected by PCR. Antibodies to recombinant SIV p27 and gp140 reached a plateau by 12 weeks and persisted (mean $\log_{10}$ ELISA 2·8 [SD 0·1] and 2·9 [0·3], respectively). Neutralising antibodies against J5 reached titres between $\log_{10}$ 1·8 and 2·7 (mean 2·1 [0·4]). At 39 weeks after infection with C8, these macaques and four control animals were challenged with 10^7 median infective doses (MID_{50}) of J5M, a cell-free stock of J5 virus, prepared in peripheral blood mononuclear cells from macaques.[3] The course of infection was assessed by virus recovery and a diagnostic PCR in which a region of *nef* is amplified and the two clones J5 and C8 can be distinguished.[4] Virus was recovered from all controls after challenge but not from the animals that had been preinfected with C8 (table). After challenge, the *nef* PCR identified proviral sequences derived from J5 in the controls. By contrast, no such sequences were detected in the blood of macaques previously infected with C8. Furthermore, no anamnestic antibody response to envelope were detected by ELISA with recombinant gp140 (Repligen)[5] in macaques infected with C8 (table).

Group	Virus recovery: co-cultivation*/PCR			Antibody titres to envelope			
	0	2	8	0	4	8	12
Cell-free virus (J5) challenge							
C8-infected							
L103	-/-	-/C8	-/C8	2·8	3·3	3·2	3·3
L104	-/-	-/-	-/C8	2·8	2·9	3·2	3·3
L105	-/C8	-/-	-/C8	3·3	3·8	3·2	3·3
L106	-/C8	-/-	-/-	2·8	2·8	3·2	2·7
Controls							
L107	-/-	+/J5	+/J5	<1·5	3·4	3·9	3·6
L108	-/-	+/J5	+/J5	<1·5	3·9	3·8	3·7
M17	-/-	+/J5	+/J5	<1·5	2·9	3·7	3·4
M18	-/-	-/-	+/J5	<1·5	<1·5	2·6	3·6

	Virus recovery: Co-cultivation*				PCR		
	0	2	4	8	0	2	8
Cell-associated virus (J82) challenge							
C8-infected							
L101	-	1	-	-	-	-	C8
L102	+	<0·2	-	-	-	-	-
M15	-	<0·2	-	-	-	C8	-
M16	-	<0·2	-	+	C8	C8	C8
Controls							
N13	-	>10³	+	+	-	J82	J82
N14	-	$10^{2.3}$	+	+	-	J82	J82
N15	-	10^{3}	+	+	-	J82	J82
N16	-	10^{2}	+	+	-	J82	J82

Times are baseline and weeks after challenge.
*+=virus recovered from 5×10^6 peripheral blood mononuclear cells. Figures indicate number of infected cells per 10^6 peripheral blood mononuclear cells cultured. -=no virus recovered.

Table: **Recovery of SIV in C8-infected and control macaques after challenge with cell-free and cell-associated virus**

All control macaques showed more than a 10-fold increase in antibodies to SIV between 0 and 8 weeks after challenge.

A further group of four macaques (L101, L102, M15, M16) were infected with 10^4 $TCID_{50}$ of the 9/90 pool of C8. The course of infection was similar to that for L103–L106. Neutralising antibody titres against 32H virus (the source of J82) reached log_{10} 1·3 to 2·4. At 49 weeks after infection with C8, all four macaques and four controls (N13–N16) were challenged with 10 MID_{50} of the cell-associated virus stock, J82. The J82 challenge comprised 23 000 spleen cells collected from a macaque 10 weeks after infection with the uncloned 32H strain of SIVmac251.[6] All controls became infected as judged by virus reisolation and confirmed by PCR (table). Virus was recovered by co-cultivation of peripheral blood mononuclear cells with C8166 cells (a human T-cell line) for up to 28 days and confirmed by a p27-antigen-capture ELISA. At 2 weeks after challenge, the number of infected cells was titrated and found to be between $10^{2.3}$ and 10^4 infected cells per 10^6 cultured peripheral blood mononuclear cells. By contrast, we did not recover J82-derived virus from those macaques that had been infected chronically with C8 virus. This was confirmed by PCR (table). After challenge with J82 virus there were no anamnestic antibody responses to SIV envelope in C8-infected animals (figure).

Our results show that chronic infection with the attenuated molecular clone C8 can protect against superinfection by not only cell-free but also by cell-associated virus challenge. The difference between the test and control groups was significant (all four of the controls became infected with J82 whereas none of the four C8-infected animals had J82 [table]). This observation is important because it provides the first evidence of virus-induced protection against virus-infected spleen cells. The relative importance of cell-associated and cell-free virus in the natural transmission of HIV-1 is uncertain. An AIDS vaccine would have to protect against both types of virus challenge. Both virus-challenge stocks we used replicated readily in macaques and reached high virus burdens. Titration of peripheral blood mononuclear cells isolated from control macaques 2 weeks after challenge with J82 indicated that between 0·1% and 1·0% of cells were infected. Our similar studies of naive macaques challenged with the J5M stock revealed that 0·01% to 0·1% of cells were infected. Nevertheless, there was no evidence for the replication of either challenge virus in macaques previously infected with C8 virus.

In the SIV/macaque model, our results and those of Daniel et al[3] demonstrate that live-attenuated virus vaccines confer potent protection against uncloned SIV. However, the use of live-attenuated HIV as an AIDS vaccine in man is fraught with problems of safety. Most important is the lifelong persistence of the attenuated virus and the integration of proviral DNA into the host genome. The clones of SIV that have been used as live virus vaccines (C8 and SIVmac239Δ*nef*[3]) contain deletions in the *nef* gene. In C8 there is an in-frame deletion of 4 aminoacids; in SIVmac239Δ*nef* a large 150 basepair frameshift truncation was engineered into the *nef* gene. Although a functional *nef* gene is important for maintenance of the virus burden of SIVmac in macaques, the mechanism of action of *nef* is unknown.[7] An in-vitro correlate for *nef* function in vivo has yet to be identified. Without this information, we cannot assess the likely stability of an attenuated virus vaccine nor the changes that lead to reversion in vivo. Because there were only seven nucleotide differences between the pathogenic J5 and attenuated C8 clones, we will be able to address this question and identify the critical differences that attenuate the virus.

Our observations also raise the question about the nature of protection that is effective against virus-infected cells injected intravenously but cannot eliminate an

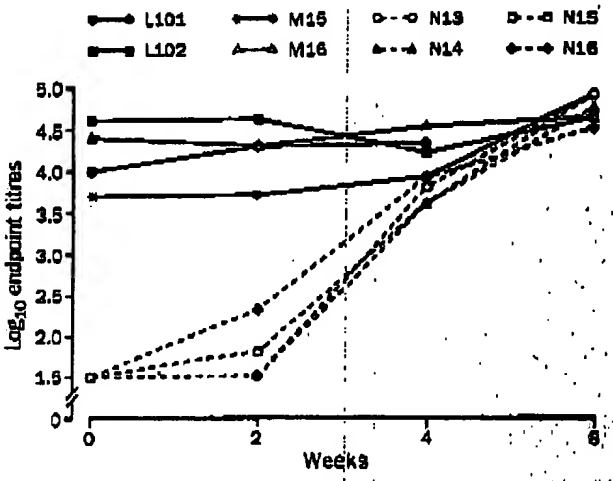

Figure: **Antibody responses to SIV envelope in plasma of macaques after challenge with cell-associated virus-challenge stock J82**

L101, L102, M15, and M16 had been infected with C8 virus 49 weeks before challenge with J82; N13–N16 were controls. Log_{10} end-point titres were determined by ELISA.

established infection. The hazards of a live-attenuated retrovirus vaccine in man would be obviated if the mechanism of protection could be identified and elicited by alternative subunit or recombinant vaccine. The protection is unlikely to be mediated by antibody. The titres of neutralising antibodies and antibodies measured by ELISA to envelope and gag proteins induced by C8 infection are at least 10 times lower than levels we have previously obtained in animals vaccinated with recombinant vaccines that failed to protect.[8] Furthermore, passive transfer of antibodies from persistently infected macaques with high titres of neutralising activity failed to prevent infection with SIV.[5] We also have evidence that the protection conferred by live-attenuated SIV is effective against challenge with a chimaeric virus in which the *env*, *tat*, and *rev* genes of SIV have been replaced by those of HIV-1, suggesting that protection mediated by live-attenuated virus vaccines is not dependent on the envelope and is targeted via a different virus protein. Cellular immunity may explain the results we have obtained. Although cytotoxic T cells were not measured in the animals in these experiments, other macaques infected with C8 virus have generated strong MHC class I restricted cytotoxic-T-lymphocyte activity, particularly against Nef protein (F Gotch and A Gallimore, Institute of Molecular Medicine, Oxford). Another possibility is that protection is mediated by an interference phenomenon similar to that described for murine retroviruses.[9]

The protection conferred by infection with live-attenuated SIV has the properties required of a successful AIDS vaccine. An identical approach could not be used in man without extensive safety investigations. Nevertheless, our results show that an effective AIDS vaccine is feasible and can prevent infection with cell-associated and cell-free virus, which Sabin believed was unattainable.[10] If the immune mechanisms that mediate this protection can be identified and reproduced by less hazardous methods, the development of safe and effective AIDS vaccines will be advanced.

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